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# Concordance of ctDNA and tissue mutations in NSCLC: A meta-analysis

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**ABSTRACT**

Systematic evaluation of the consistency between circulating tumor DNA (ctDNA) in plasma and tumor tissue samples in mutations in non-small cell lung cancer (NSCLC) patients. To collect publicly published literature from numerous important international medical databases through computer retrieval. To compare the differences in literature data related to gene mutations between plasma ctDNA and tumor tissue samples, a meta-analysis was performed using Stata 12.0 software while taking into account the inclusion and exclusion criteria. This article includes a total of 15 research data and collected data reports from 15 groups of NSCLC patients. The results are displayed using tissue samples as the gold standard. The Pearson correlation coefficients of sensitivity and specificity were used to calculate rho=0.044, and P=0.893. The Q-test found poor homogeneity and high heterogeneity in sensitivity and specificity among research data from various literature studies ($I^2$>50%, P<0.1). The area under the SROC curve is 0.97 (95% *CI*: 0.96~0.99). The small sample subgroup has high heterogeneity, and the combined diagnosis effect size is 26[6~111], lower than the large sample subgroup 185320 [0~$2.7\times10^{12}$]. When taking 200 as the cut-off point, the combined effect size of the small sample subgroup is 46 [12~183], still lower than that of the large sample subgroup 429 [52~3574]. From this, it can be concluded that the consistency of small-sample studies is higher than the quality of large-sample studies, and the heterogeneity is relatively low. From the perspective of mutation types, the heterogeneity of literature with EGFR gene mutations alone is higher than that of literature with non-EGFR mutations alone, and the consistency is lower. The consistency of using plasma ctDNA to detect mutations in NSCLC patients with tumor tissue samples is influenced by the type of mutation gene and sample size measured by the patient, and there is a significant bias in related studies.

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## Introduction

It is an important component of free nucleic acid in plasma. Quantitative and qualitative changes in this marker are used to identify and track all types of cancer, prenatal diagnosis, cardiovascular disease and organ transplantation. The source of Cell-Free DNA (cfDNA) in the plasma of healthy people, mainly comes from apoptosis, although studies have shown that living cells may also actively release DNA fragments into the plasma. Circulating tumor DNA (ctDNA) is a subset of cfDNA that is secreted into the blood by cancer cells and tumors. Most of the DNA is located in the nucleus of the cell, and as the tumor grows, the cells die and are replaced by new cells. Dead cells are broken down and their contents (including DNA) are released into the blood. ctDNA is single- or double-stranded DNA released into the blood by tumor cells and therefore contains original tumor mutations. In recent years, liquid biopsy based on ctDNA analysis has greatly facilitated the molecular diagnosis and monitoring of cancer. Studies have shown that screening for genetic mutations using ctDNA is highly sensitive and specific, suggesting that ctDNA analysis may significantly improve current tumor detection systems and even aid in early diagnosis. In addition, ctDNA analysis can accurately judge tumor progression, prognosis and assist targeted therapy (1-3).

In patients with Non-small Cell Lung Cancer (NSCLC), high levels of tissue Tumor Mutation Burden (tTMB) or blood Tumor Mutation Burden (bTMB) are associated with immune therapy response (1). It can be seen that immunotherapy has become a new trend to replace existing treatment plans. For immunotherapy, different driving genes, different subtypes of driving genes, or co-mutations can all have an impact on clinical efficacy (2). Liquid biopsy, especially circulating tumor DNA (ctDNA) analysis, has been applied in clinical practice as a new non-invasive method for the diagnosis and monitoring of NSCLC. For a long time in the past, with the implementation of multiple targeted gene therapy regimens for NSCLC treatment, the traditional method of Next-generation Sequencing (NGS) from tissue biopsy samples has gradually evolved into plasma-based ctDNA. It is also known as liquid biopsy, which supplements tissue biopsy methods and provides guidance for first-line treatment (3). Most ctDNA is released by apoptotic or necrotic tumor cells, which can reflect the genetic characteristics of tumors. Numerous studies have reported high consistency in mutation profiles from liquid biopsy and tissue biopsy, particularly in terms of driving genes (4). However, the sample size of most studies is small, and there is still controversy over the consistency of results between plasma and tissue samples

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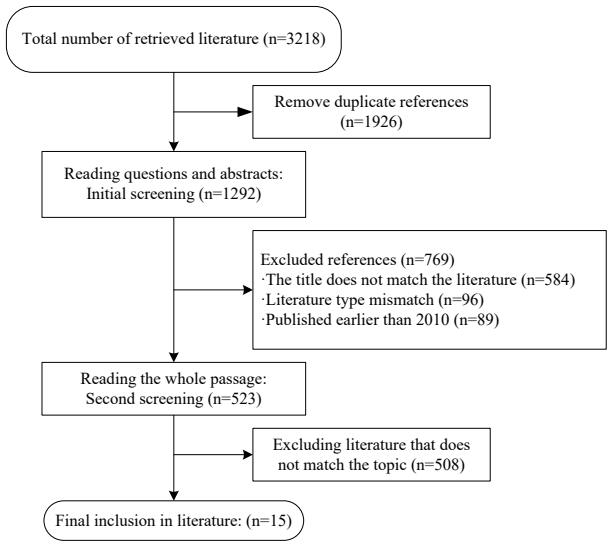


**Figure 1.** Literature Retrieval Strategy.

for different gene mutation types in NSCLC patients. This study aimed to use meta-analysis to determine the consistency of plasma ctDNA and tumor tissue samples in different gene mutations in NSCLC patients. The results are reported as follows.

## Materials and Methods

### Inclusion and Exclusion Criteria

#### *Inclusion Criteria*

Research type: cohort study; Publication language: Chinese or English for public publication of literature; Research subjects: NSCLC patients; Exposure factors: plasma ctDNA and tumor tissue samples were tested; Outcome indicators: whether the patient has experienced a genetic mutation event and what type of genetic mutation has occurred.

#### *Exclusion criteria*

Communication or meeting minutes; Repeated publication of literature; After reading the entire text, it was found that it was unrelated to the topic; Incomplete data description or inability to obtain the full text.

### Retrieval Strategy

Computer searches were conducted on the Chinese Biomedical Literature Database (CBM), Embase, PubMed, Medline, Cochrane Online Library, CNKI, Wanfang, and the National Comprehensive Cancer Network (NCCN) of the United States. The study on the relationship between gene mutations in NSCLC patients and plasma ctDNA and tumor tissue samples, which was publicly published from January 2010 to March 2023, was collected. Search term: Non-small cell lung cancer, mutation, gene, plasma, tissue specimens, plasma circulating tumor DNA, ctDNA. The retrieval process was completed by two researchers in the research group, using cross-checking to correct the research data, and utilizing "https://www.connectedpapers.com/" to track the references included in the website and establish a database (Figure 1).

### Quality evaluation

The included studies were all ctDNA testing of plasma and tissue specimens. Table 1 shows the quality evaluation criteria for the literature collected and included in this study based on the QUADAS statement.

### Statistical analysis

Stata 15.0 software was used for statistical analysis, and SMD and 95% *CI* were used to analyze the data of each group. Comparing the differences in ctDNA examination results between plasma and tissue specimens, the correction level is $\alpha = 0.05$. To test the consistency between studies, $I^2=0$ indicates that heterogeneity is not statistically significant; $I^2=50\%$ is moderate heterogeneity; $I^2>50\%$ indicates significant heterogeneity. When there is no statistical heterogeneity between studies, fixed model effects were used; Otherwise, a random effects model would be adopted. Drawing funnel plots, Begg rank correlation tests, and Egger linear regression methods to test whether there is publication bias in the results between each study. Using sensitivity to analyze the stability of detection results and evaluate them, and conducting subgroup analysis based on sample size and mutation source.

## Results

Basic information on included literature has been shown in Table 2.

### Inclusion in literature quality evaluation

The overall quality of the included studies is average,

**Table 1.** Evaluation Indicators and Methods for Literature Quality.

| Serial number | Items | Content | Answer |
|---|---|---|---|
| 1 | Included disease spectrum | Representative examination population in clinical practice | Yes/No |
| 2 | Research object | Clear selection criteria | Yes/No |
| 3 | Interval time | The reference test and evaluation test Detection are short enough | Yes/No |
| 4 | Reference test | All samples accepted | Yes/No |
| 5 | Reference standard test | Independent of the test to be evaluated | Yes/No |
| 6 | Interpretation of the results of the test to be evaluated | Conduct without knowing the reference test results | Yes/No |
| 7 | Interpretation of results from reference standard tests | Conduct without knowing the results of the test to be evaluated | Yes/No |
| 8 | Intermediate test results | Difficult to explain and report | Yes/No |
| 9 | Case situation | All cases reported | Yes/No |

**Table 2.** Included basic information in the literature.

| Reports | Respondent | ctDNA method | Mutant Gene |
|---|---|---|---|
| Desmeules et al. (5) | Late NSCLC | NGS | EGFR, ALK |
| Zhang et al. (6) | NSCLC in stage III/IV | ADx-ARMS | EGFR |
| Li et al. (7) | NSCLC | RT-PCR | TP53, EGFR, PIK3CA |
| Park et al. (8) | NSCLC | RT-PCR | EGFR, ALK, ROS1 |
| Mack et al. (9) | Adenocarcinoma of lung in stage IIIB-IV or NSCLC | Hudrangt360 | EGFR, MET, BRAF |
| Chen et al. (10) | NSCLC in stage I-IV | NGS | EGFR, PIK3CA, KRAS |
| Usui et al. (11) | Progressive or postoperative recurrence of NSCLC | Peptide nucleic acid locking nucleic acid blocking method | T790M |
| Mirtavoos-Mahyari et al. (12) | NSCLC | Polymerase chain reaction | EGFR |
| Yang et al. (13) | NSCLC | Targeted depth sequencing | EGFR, KRAS |
| Veldore et al. (14) | Late NSCLC | Isothermal amplification PCR | EGFR |
| Roosan et al. (15) | NSCLC | Guardant Health | BRAF |
| Thompson et al. (16) | NSCLC | NGS | EGFR |
| Long et al. (17) | NSCLC | ARMS-PCR test | EGFR |
| Xu et al. (18) | NSCLC in stage II-IV | ARMS-PCR | EGFR, ROS |
| Guo et al. (19) | NSCLC patients in stages I-III | Real-time PCR | EGFR |

**Table 3.** Quality evaluation results of included literature.

| Reports | Representative disease spectrum | Define sample selection | Interval time of detection | All accepted for reference | Independence of experiments | blind trials | blind measurement reference trials | Report all test results | Report all cases |
|---|---|---|---|---|---|---|---|---|---|
| Desmeules et al. (5) | Yes | Yes | Not sure | Yes | Yes | Not sure | Not sure | Yes | Yes |
| Zhang et al. (6) | Yes | Yes | have | Yes | Yes | Yes | No | Yes | Yes |
| Li et al. (7) | Yes | Yes | have | Yes | Yes | No | No | Yes | Yes |
| Park et al. (8) | Yes | Yes | have | Yes | Yes | No | No | Yes | Yes |
| Mack et al. (9) | Yes | Yes | Not sure | Yes | Yes | No | No | Yes | Yes |
| Chen et al. (10) | Yes | Yes | have | No | Yes | Yes | No | Yes | Yes |
| Usui et al. (11) | Yes | Yes | have | No | Yes | No | No | Yes | Yes |
| Mirtavoos-Mahyari et al. (12) | Yes | Yes | have | Yes | Yes | Yes | No | Yes | Yes |
| Yang et al. (13) | Yes | Yes | Not sure | Yes | Yes | Yes | No | Yes | Yes |
| Veldore et al. (14) | Yes | Yes | have | Yes | Yes | No | No | Yes | Yes |
| Roosan et al. (15) | Yes | Yes | have | Yes | Yes | No | No | Yes | Yes |
| Thompson et al. (16) | Yes | Yes | Not sure | No | Yes | No | No | Yes | Yes |
| Long et al. (17) | Yes | Yes | have | Yes | Yes | No | No | Yes | Yes |
| Xu et al. (18) | Yes | Yes | have | Yes | Yes | No | No | Yes | Yes |
| Guo et al. (19) | Yes | Yes | have | Yes | Yes | No | No | Yes | Yes |

as shown in Table 3. The vast majority did not design blind trials or blind measurement reference trials, and some trials did not provide detailed descriptions of detection intervals.

## Comparison of methodological results

### *Comparison of mutation results between plasma ctDNA and tumor tissue specimens*

A total of 15 NSCLC patient data reports were collected from 15 included studies, and the results were shown using tissue specimens as the gold standard (Table 4 and Figure 2). The Pearson correlation coefficients of sensitivity and specificity were used to calculate rho=0.044, and P=0.893, confirming that there is no threshold effect between studies and that sensitivity and specificity can be combined. The combined sensitivity, specificity, positive likelihood ratio, negative likelihood ratio, and accuracy probability ratio of plasma ctDNA in the random effect model [95% *CI*] were 0.68[0.51~0.81], 0.99[0.98~1.00], 75.7[25.7~222.8], 0.32[0.20~0.52], 236[62~893]. After the Q test, it was proven that the homogeneity and heterogeneity of sensitivity and specificity among the research data in various literature were poor ($I^2$>50%, P<0.1) (Figure 3). The area under the SROC curve is 0.97(95% *CI*: 0.96-0.99) (Figure 4).

**Table 4.** Comparison of mutation results between plasma ctDNA and tumor tissue samples.

| Reports | *n* | Plasma ctDNA | | Sample standards | |
|---|---|---|---|---|---|
| | | positive | negative | positive | negative |
| Desmeules et al. (5) | 91 | 43 | 48 | 50 | 41 |
| Zhang et al. (6) | 1001 | 189 | 812 | 251 | 750 |
| Li et al. (7) | 50 | 23 | 27 | 48 | 2 |
| Park et al. (8) | 287 | 135 | 152 | 174 | 113 |
| Mack et al. (9) | 8388 | 7214 | 1174 | 7851 | 537 |
| Chen et al. (10) | 50 | 22 | 28 | 50 | 0 |
| Usui et al. (11) | 66 | 22 | 44 | 33 | 33 |
| Mirtavoos-Mahyari et al. (12) | 20 | 18 | 2 | 20 | 0 |
| Yang et al. (13) | 99 | 21 | 78 | 59 | 40 |
| Veldore et al. (14) | 132 | 41 | 91 | 45 | 87 |
| Roosan et al. (15) | 370 | 74 | 296 | 177 | 193 |
| Thompson et al. (16) | 50 | 45 | 5 | 50 | 0 |
| Long et al. (17) | 315 | 138 | 177 | 205 | 110 |
| Xu et al. (18) | 34 | 32 | 2 | 32 | 2 |
| Guo et al. (19) | 174 | 6 | 168 | 27 | 147 |

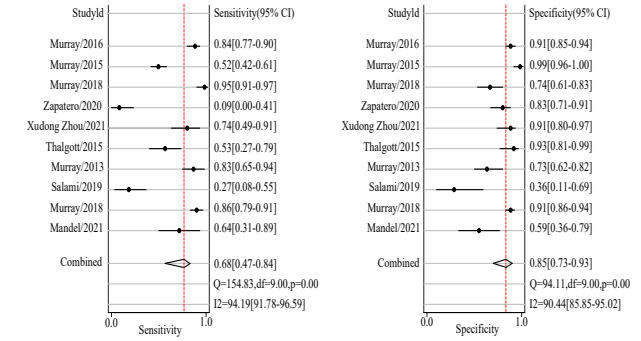


**Figure 2.** Forest graphics of mutations in plasma ctDNA and tumor tissue specimens.

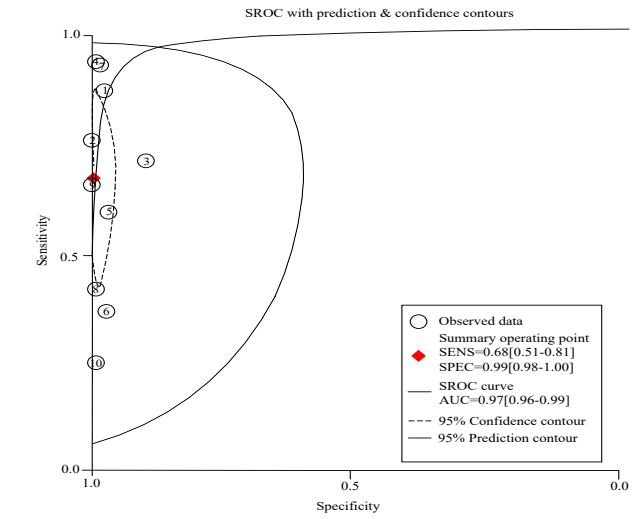


**Figure 3.** SROC curve analysis.

## Sensitivity analysis

In the subsequent analysis, the sensitivity and specificity of each study were summarized using the stepwise exclusion method, and the combined sensitivity and specificity would gradually decrease. The stability of the included literature was good.

## Subgroup analysis

The sample size is arranged from low to high, and the significant changes are used as the grouping boundary point. The boundary point for this study is 100. The result after grouping is that there is high heterogeneity in the small sample subgroup. The combined diagnostic effect size was 26[6~111], lower than 185320[0~$2.7\times10^{12}$] in large sample subgroups. When 200 is the cut-off point, the combined effect size of the small sample subgroup is 46[12~183], which is still lower than that of the large sample subgroup 429[52~3574]. Conclusion: Large sample studies exhibit greater heterogeneity. For mutation types, the heterogeneity of simple EGFR gene mutation literature is higher than that of non-simple EGFR mutation literature. The consistency between plasma ctDNA and tissue specimen examination results may be affected by differences in mutation gene types (Table 5).

## Publication bias detection

Deek's funnel diagram was used to evaluate the risk of bias in the literature included in this study. From the results, it can be seen that there is poor symmetry (P=0.00) among various studies, and there is significant bias in the literature (Figure 4).

## Discussion

The pathogenesis of NSCLC is closely related to gene mutations, making EGFR mutation/ALK fusion molecular detection a fundamental diagnostic and treatment method for NSCLC in China. With the widespread popularity of high-throughput sequencing technology, gene screening technology and DNA mutation identification have gradually replaced biopsy due to their unique advantages of good repeatability, small damage and dynamic monitoring. Repeated biopsy is used to determine the genetic evolution of patients, which is an invasive examination that requires accurate and appropriate sampling, and is prone to abnormal results due to the heterogeneity within the tumor. In this way, tissue specimen examination is not very suitable for molecular analysis. Plasma ctDNA examination is a

**Table 5.** Subgroup analysis results are included in the literature.

| Subgroup classification | Grouping criteria | Number of articles | $I^2$ | *P* | OR (95%CI) |
|---|---|---|---|---|---|
| sample size | *n*<100 | 8 | >50% | <0.001 | 26 [6~111] |
| | *n*≥100 | 7 | >50% | <0.001 | 185320 [0~2.7×10[12]] |
| | *n*<200 | 10 | >50% | <0.001 | 46[12~183] |
| | *n*≥200 | 5 | >50% | <0.001 | 429[52~3574] |
| Mutation type | simple EGFR mutation | 6 | >50% | <0.001 | 201 [33~1239] |
| | Non-simple EGFR mutation | 9 | >50% | <0.001 | 62 [11~332] |

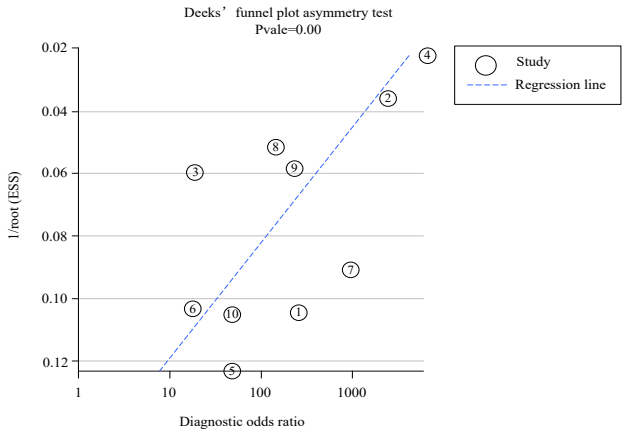


**Figure 4.** Deek's Funnel Diagram.

technique that utilizes circulating DNA fragments carrying tumor-specific sequence changes and extracts them from the blood, which can serve as a marker for total circulating DNA. Non-invasive techniques based on blood samples have great potential in NSCLC patients with EGFR mutations (20). Liquid biopsy can non-invasive detect multiple targeted genomes, guiding clinical targeted therapy directions. At the same time, it can monitor changes such as gene mutations and drug resistance, overcoming spatial and temporal heterogeneity. This assists in establishing management strategies for different stages of NSCLC patients' diseases, such as screening and minimum residual lesion detection. This also provides ideas for guiding adjuvant therapy, early detection of recurrence, initiation and response monitoring of systemic therapy (targeted or immunotherapy), and drug resistance gene typing (21). It shows that both plasma ctDNA and tissue specimen examination can assist in mutation detection in NSCLC patients.

In the era of personalized medicine, detecting more and more predictive biomarkers is becoming a top priority. However, the tissue biopsies of these patients are often insufficient to meet routine treatment requirements, which results in their inability to obtain the clinical benefits of biomarker therapy. By analyzing the DNA sequence in tissue samples, multiple gene mutations can be detected, which can help doctors determine whether patients have specific disease risk factors and guide the development of treatment plans. Mutation analysis of plasma ctDNA demonstrates the potential for disease monitoring in various cancers. EGFR mutation detection based on ctDNA is a monitoring tool for NSCLC patients, and EGFR mutation patients can serve as prognostic markers for first-line treatment (22). In this way, to solve the problem of not being able to obtain biomarkers promptly, next-generation sequencing technology (NGS) has become crucial. In fact, different NGS systems can simultaneously detect several clinically relevant low-frequency hot spot mutations in one operation (23). The presence of EGFR mutations in ctDNA can predict the response of EGFR TKIs (24). With the development of the NGS system, the results of plasma ctDNA and tissue sample DNA technology will change accordingly.

The 15 studies included in this paper collected data reports from 15 groups of NSCLC patients, and the results were exhibited using tissue specimens as the gold standard. Each group has no correlation, poor homogeneity, and high heterogeneity. The area under the SROC curve is 0.97(95% CI: 0.96-0.99). The small sample subgroup has high heterogeneity, and the combined diagnosis of 26 [6-111] effect size is lower than the large sample subgroup 185320 [0-2.7×1012]. By the time taking 200 as the cut-off point, the effect size of 46 [12-183] in the small sample sub-group is still lower than that of 429 [52-3574] in the large sample sub-group. As a result, the consistency of small-sample studies is higher than that of large-sample studies, and the heterogeneity is relatively low. Among the mutation types, the heterogeneity of simple EGFR gene mutation literature is higher than that of non-simple EGFR mutation literature, with lower consistency. In the study, not only EGFR mutations were observed, but some studies also observed mutations such as ALK and ROS1. The heterogeneity of tumors, low abundance of mutations, and the stage of the disease are related. NSCLC patients with EGFR mutations who receive EGFR tyrosine kinase inhibitors (TKIs) treatment will develop resistance to the T790M mutation (25). Detecting EGFR T790M mutations in tumor tissue is challenging. Hence, for patients with high heterogeneity of tumors, low abundance of mutations, and advanced diseases, plasma ctDNA retesting can be chosen to avoid clinical missed diagnosis.

In summary, the consistency of using plasma ctDNA and tumor tissue specimens to determine mutations in NSCLC patients is influenced by the type of mutation gene and sample size measured by the patient, and the relevant research has a significant bias.

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